Strategy and Application of Introgression Breeding in Lentil (*Lens culinaris* Medikus)

Presenter: Li-An Chen

Pulse Crop Research Group

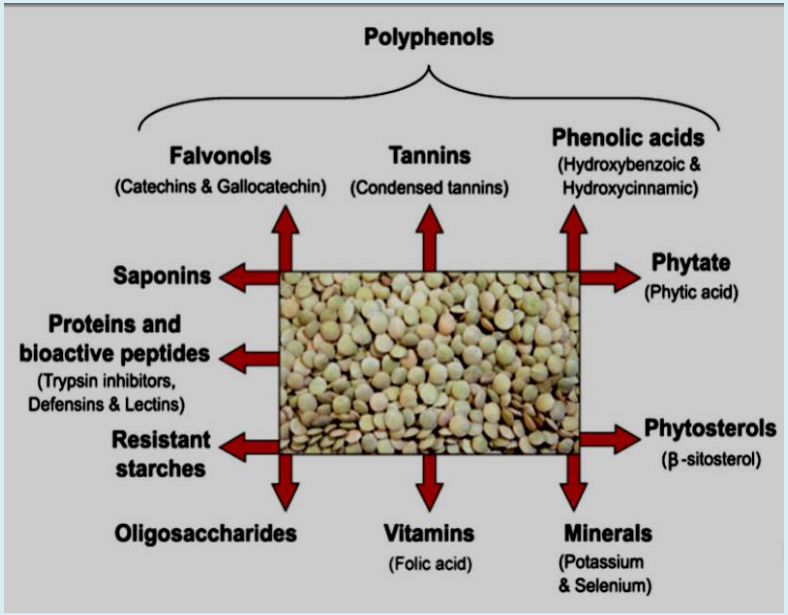
Department of Plant Sciences

University of Saskatchewan

- Canada is the world's biggest exporter of lentil.
- Catching more attention as a wholesome food.
- High nutrition value
- Bio-active Components
  - Improving colonic function, decreasing risk factors associated with obesity and metabolic syndrome

	Potato	Chickpea	Lentil	Green Pea	n [1]
Energy (kcal/100 g) [2]	299.8	282.7	263.7	245.2	
Moisture (%) [3]	7.89 ± 0.10 [a]	7.03 ± 0.17 [b]	5.66 ± 0.05 [d]	6.30 ± 0.08 [c]	3
Protein (%)	5.4 ± 0.2 [d]	18.3 ± 0.3 [c]	22.7 ± 0.2 [a]	21.3 ± 0.5 [b]	4
Lipids (%)	0.24 ± 0.06 [c]	4.97 ± 0.23 [a]	0.70 ± 0.08 [b]	0.60 ± 0.08 [b]	3
Ash (%)	3.76 ± 0.15 [a]	2.79 ± 0.07 [b]	2.62 ± 0.06 [b]	2.74 ± 0.23 [b]	3
Digestible starch (%)	68.0 ±1.3 [a]	40.2 ± 1.2 [bc]	42.6 ± 1.0 [b]	39.2 ± 1.3 [c]	6
Sugars (%)	0.99 ± 0.00 [c]	2.86 ± 0.56 [a]	1.71 ± 0.04 [b]	3.00 ± 0.36 [a]	6
Total dietary fibre (%)	7.8 ± 0.6 [c]	26.2 ± 2.7 [ab]	21.9 ± 1.4 [b]	27.1 ± 1.6 [a]	4
Resistant starch (%)	0.78 ± 0.02 [d]	4.44 ± 0.16 [c]	4.75 ± 0.14 [b]	5.17 ± 0.07 [a]	9
Dietary fibre (%)	7.0 ± 0.6 [c]	19.9 ± 2.5 [a]	14.6 ± 1.1 [b]	18.4 ± 1.0 [ab]	3

(Tosh et al., 2013)



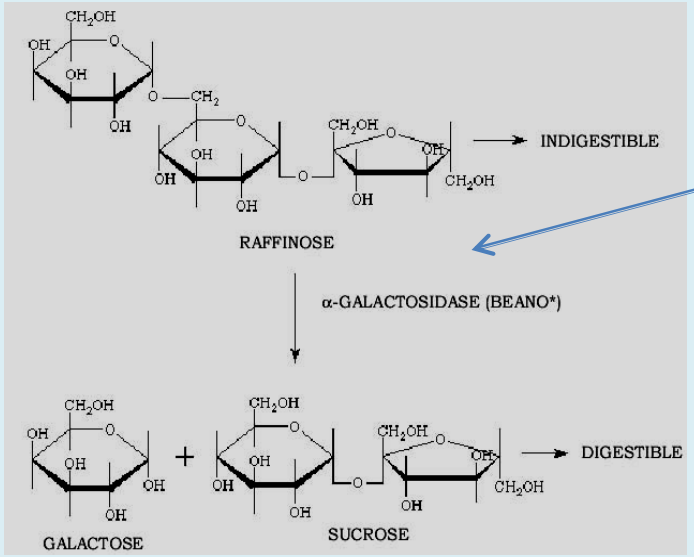
Issa et al., 2013

Legumes	Sucrose	Oligosaccharides as (%) of total sugar
Lentils	2.97±0.16[a]	46.14
Chickpeas	6.74±0.80[c]	24.97
Red beans	4.82±1.18[b]	31.70
Common white beans	4.70±0.85[b]	33.03
Great white beans	6.49±0.34[c]	32.03
Faba beans	4.38±0.20[b]	37.22

Rich in oligosaccharides, mainly raffinose family oligosaccharides (RFOs).

RFOs

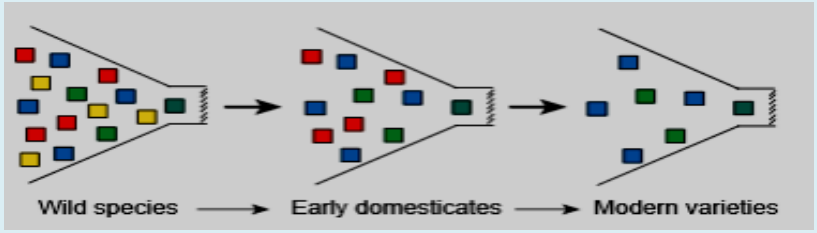
- Anti-nutrients: non-digestible, leads to flatulence and other physiological discomforts.
- A major barrier of lentil consumption
- Controlling of anti-nutrients
  - Supplements and processing: heating, soaking, de-hulling, fermentation--- negative effect



- Using breeding to reduce RFOs?

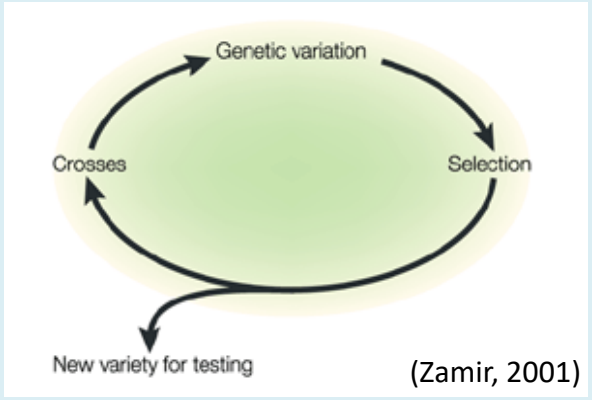
Major challenge in lentil breeding

- One of the oldest crops.



(Tanksley and McCouch, 1997)

- Founder effect: loss of allele diversity during domestication process.
- Creating genetic variation: the foundation of crop improvement cycle.



(Zamir, 2001)

Introgression breeding

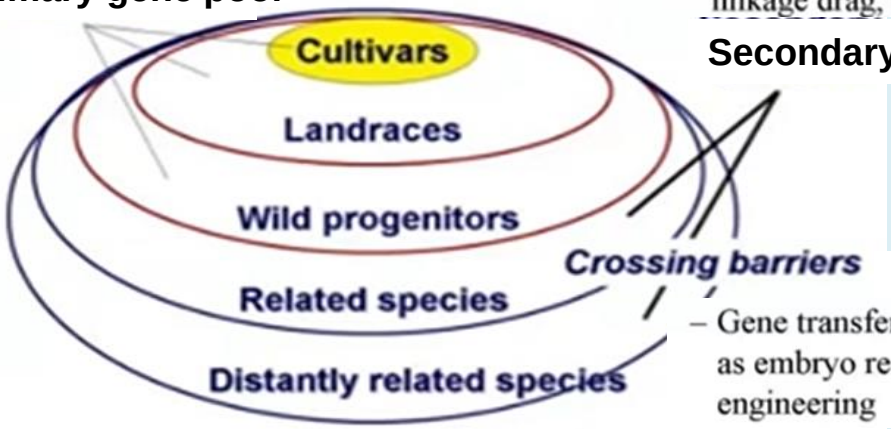
- Increasing breeding value by expanding the genetic base.
- Using exotic germplasm to transfer desirable traits to a commercially elite variety

– Genes that can be readily transferred through crossing to produce fertile F_1 hybrids (old varieties, landraces etc.)

Primary gene pool

– Plants that may be crossed with some difficulty, such as wild/weedy relatives. Problems with linkage drag, F_1 fertility.

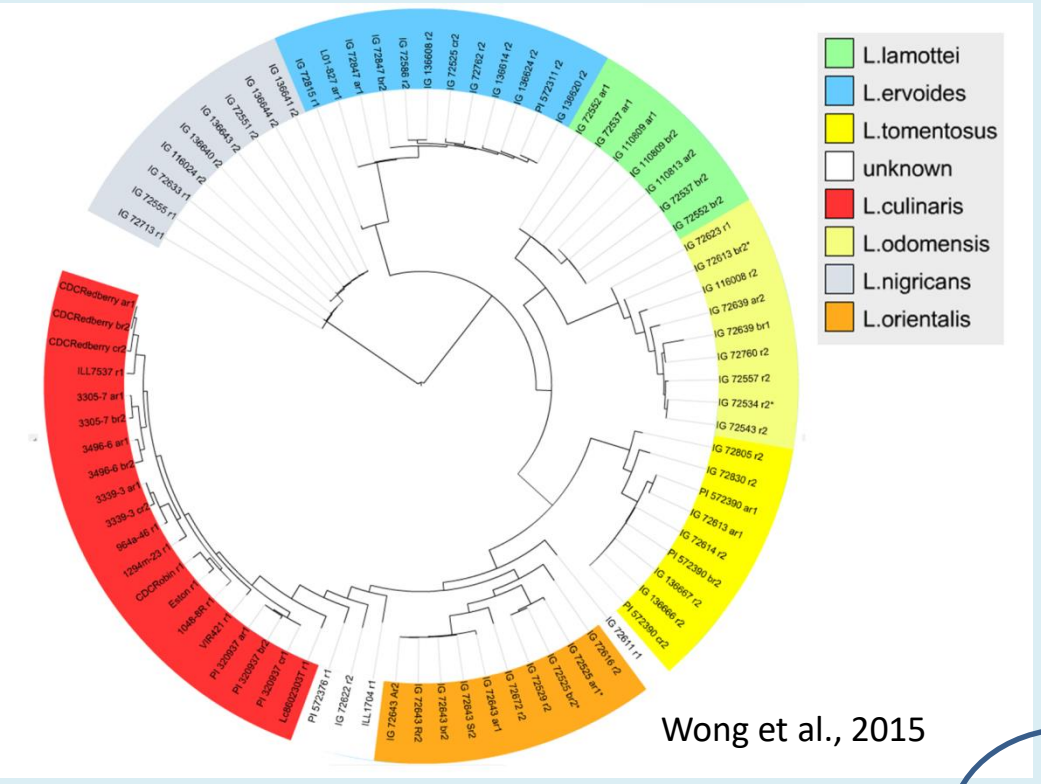
Secondary gene pool



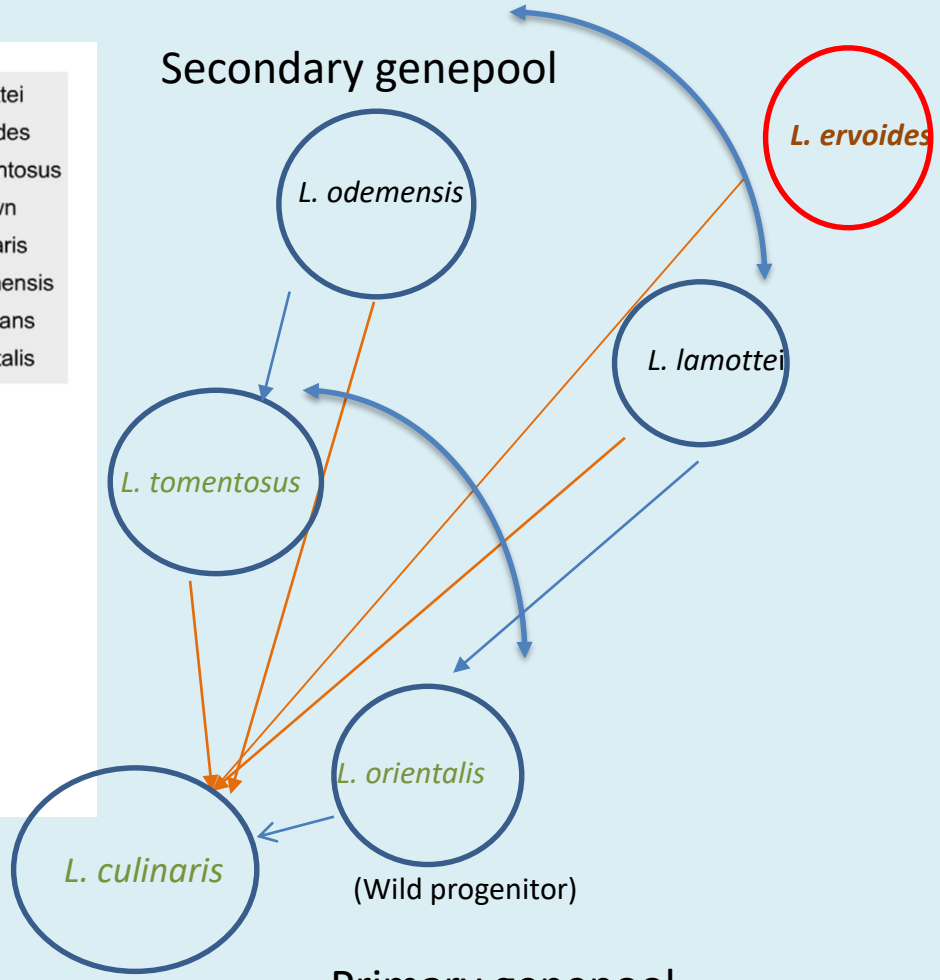
Tertiary genepool

– Gene transfer requires special techniques, such as embryo rescue, tissue culture, genetic engineering

- Lens genus : 6 taxa
- Germplasm collection



Wong et al., 2015



RFO and sucrose concentrations in wild lentil seeds (Modified from Tahir, 2011)

Latin name	Sucrose (g 100 g^{-1}flour)	TRFO (mmoles 100 g^{-1} flour)	Verbascose	Stachyose	Raffinose
			(g 100 g^{-1}flour)		
L. odemensis	0.7 - 1.4	2.8 - 4.7	0.8 - 0.9	1.7 - 2.6	1.8 - 2.6
L. c. ssp. orientali	0.4 - 0.7	3.7 - 4.1	0.5 - 0.9	2.1 - 2.3	2.5
L. ervoides	0.1 - 0.4	1.7 - 3.4	1.2 - 1.7	1.5 - 2.2	1.5 - 2.3
L. lamottei	0.4 - 0.6	2.2 - 3.2	0.9 - 1.0	1.6 - 1.9	2.0 - 2.3
L. nigricans	0.3 - 0.9	2.4 - 3.4	0.3 - 0.9	1.6 - 2.1	2.2 - 3.0
L. culinaris	0.6 - 2.7	4.0 - 6.1	1.5 - 3.0	1.5 - 3.5	1.1 - 1.6

- *L. ervoides*: the potential genetic donor
- Wlid species
  - Overcome crossing barriers
  - Phenotyping both positive and negative traits
  - Speed up the breeding procedure
    - Marker-assisted selection

The interspecific *L.c.* **L. e.* crosses

L. culinaris
(Variety: Eston)

*

L. ervoides
(Accession: IG72815)

Cross barrier

Embryo rescue

*

L. ervoides
(Accession: L01-827A)

LR-26

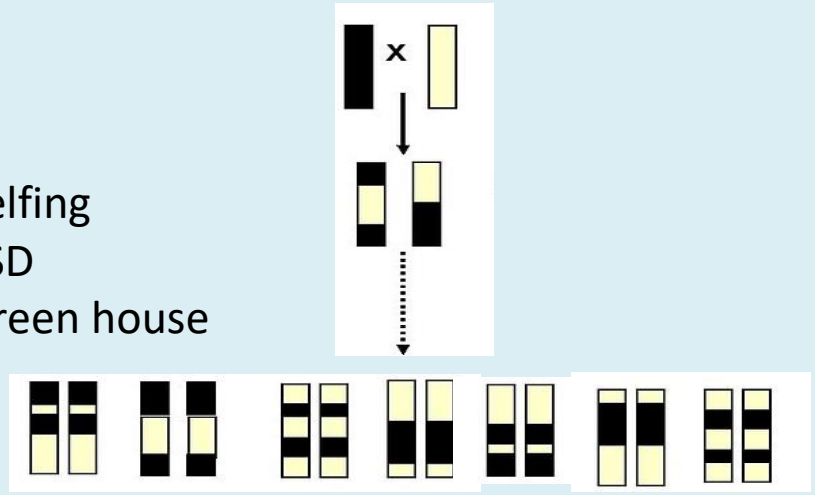
Photo courtesy:
Shyamali Saha

LR-59

	Total RFO	Raffinose	Stachyose	Verbascose
	(Enzyme-based)	(HPAEC-PAD)		
	(mMoles/100 g)	(mg/100g)		
LR-26				
Eston (*L.c.*)	4.54a	70	252	73
IG 72815 (*L.e.*)	2b	70	192	158
LR-59				
Eston (*L.c.*)	5.03a	84	321	108
L01-827 A (*L.e.*)	2.85 b	84	217	207

- Mapping introgression: RILs populations

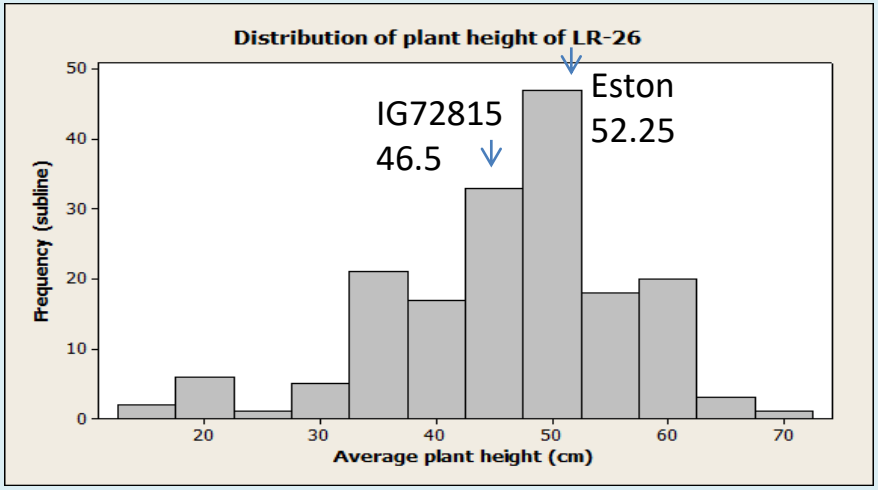
Selfing
SSD
Green house

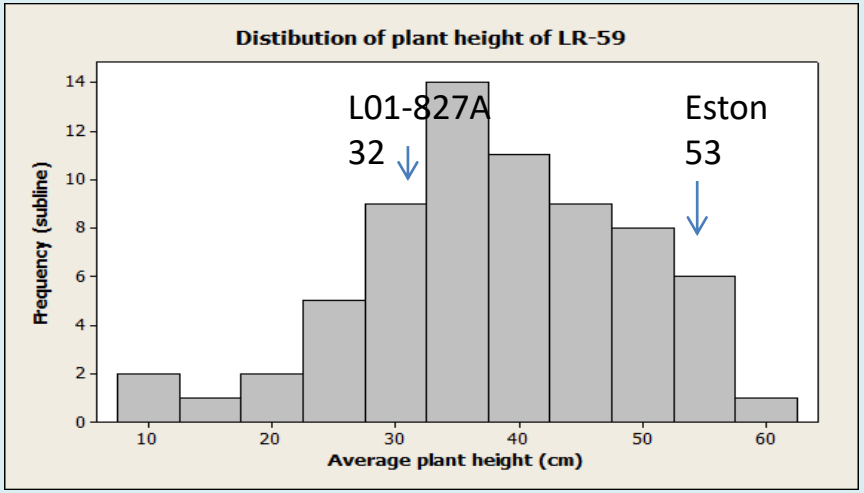


LR-26
173 RILs plus parents,
F8:12 SSD derived

LR-59
66 RILs plus parents,
F7:12 SSD derived

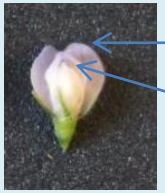
Morphological segregation



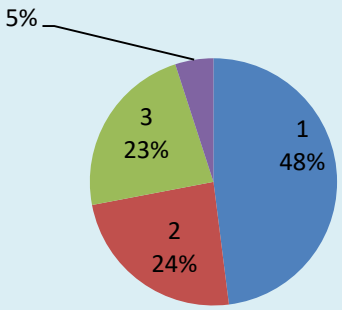


(*L.c.*)

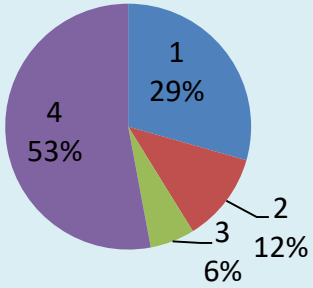
(*L.e.*)



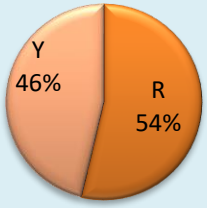
LR-26 Flower color



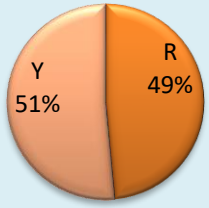
LR-59 Flower color



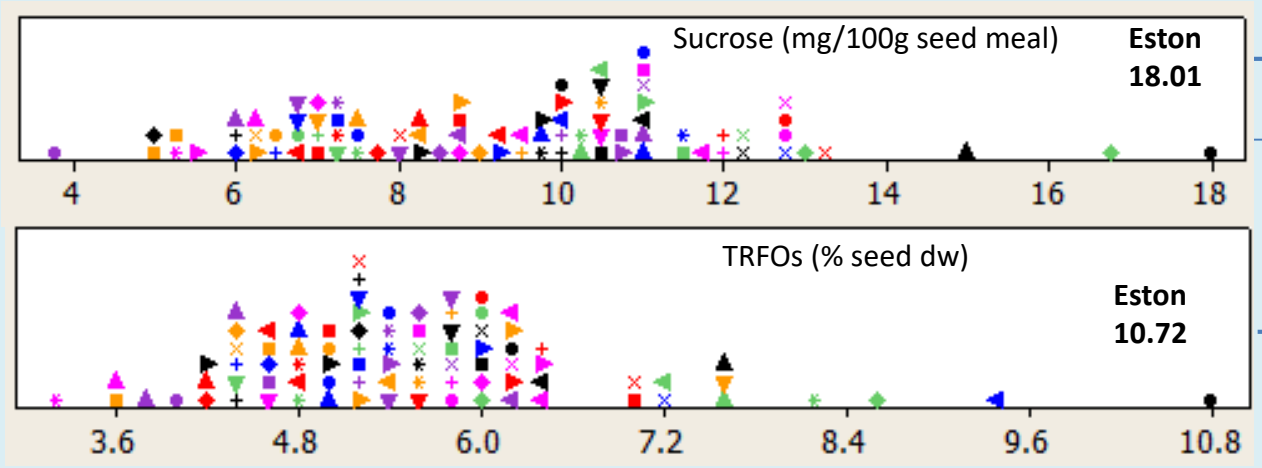
LR-26 Cotyledon color



LR-59 Cotyledon Color

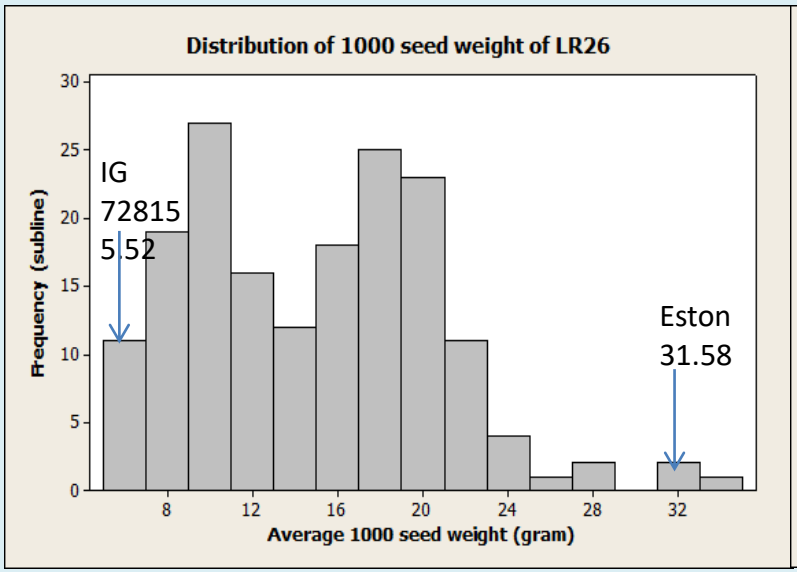


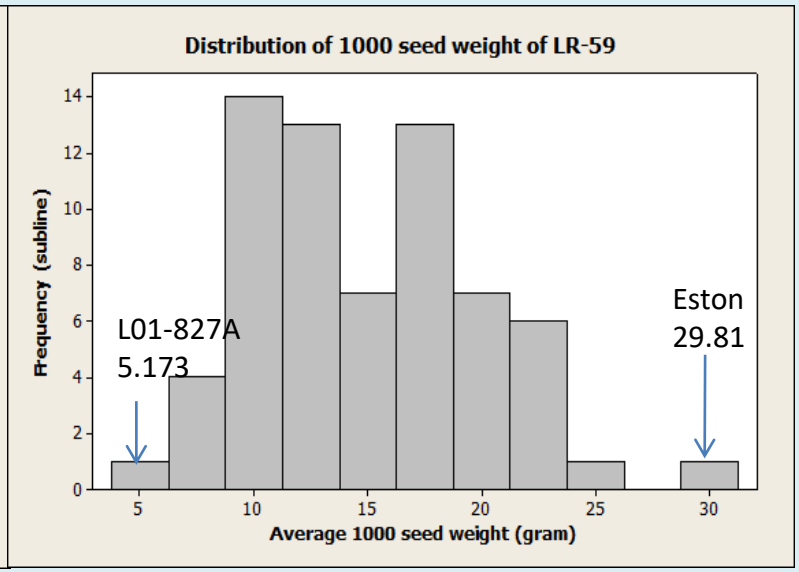
Potential for grain quality improvement



Source	DF	Mean Square Sucrose	Mean Square TRFO
Genotype	85	19.82**	4.07**
Residual		1.70	0.29
Residual df		163	164
Corrected total		249	250
CV		14.07	9.74

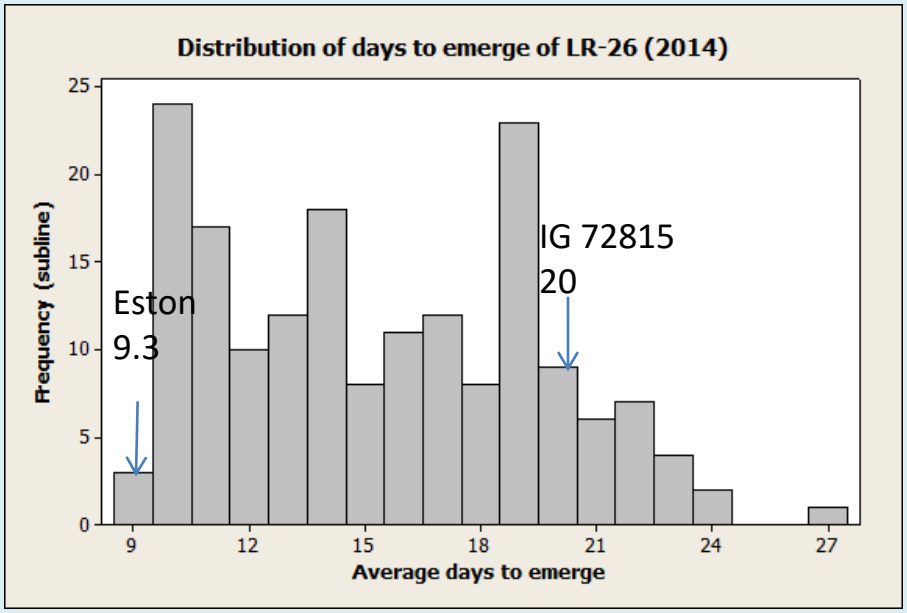
**Significant at $P \leq 0.01$.

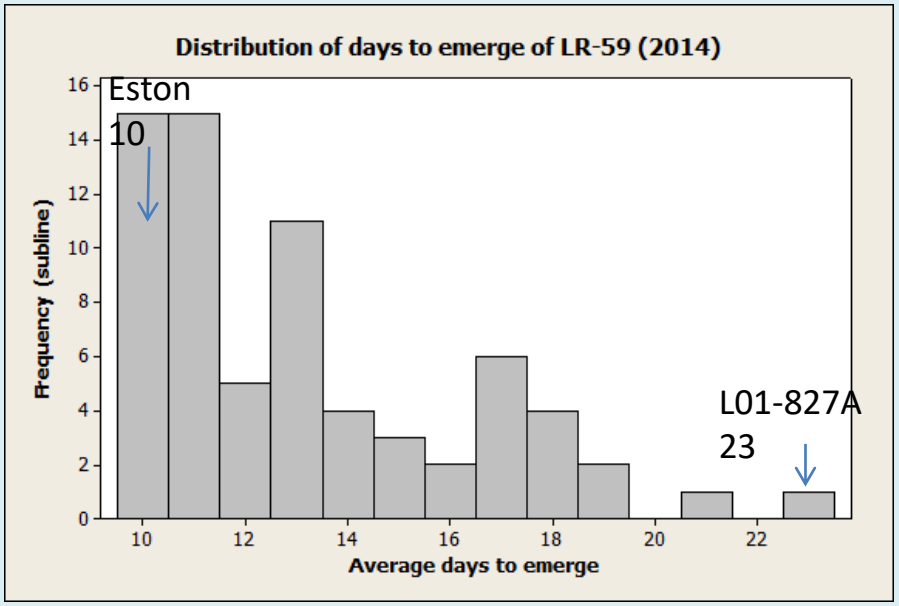


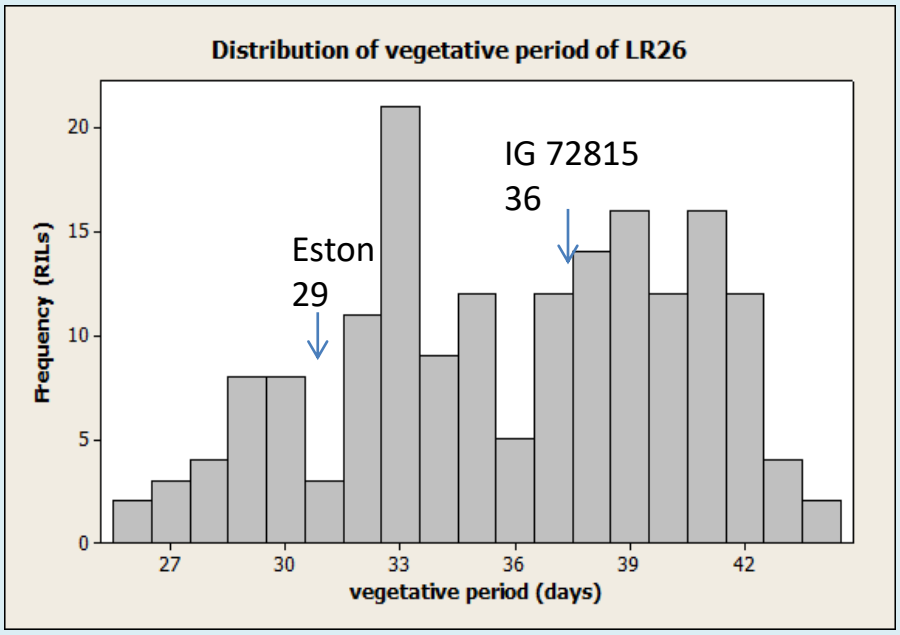


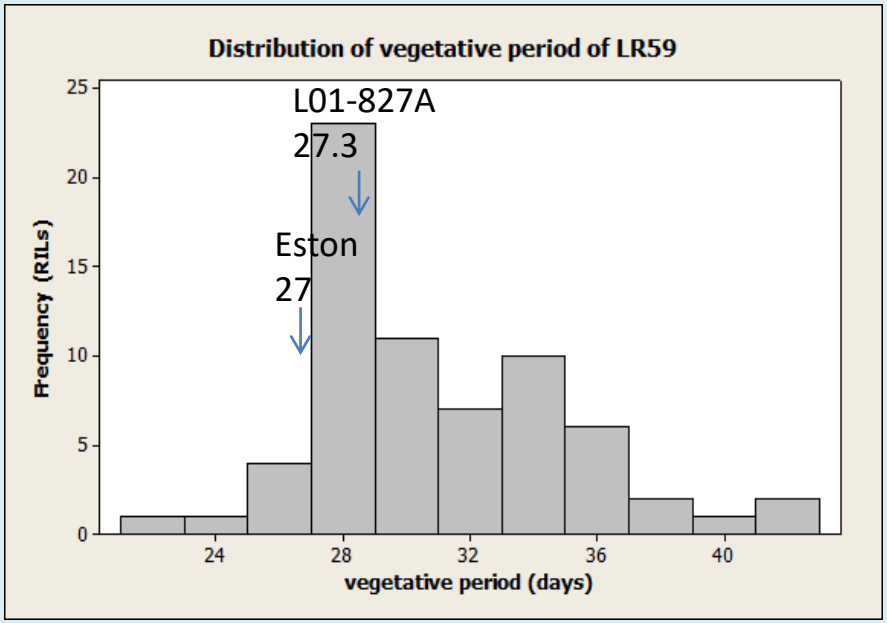
Variable	N	Mean	MSE	StDev	Min	Q1	Med	Q3	Max
TSW LR26	172	23.05	1.53	20.09	5.17	7.73	16.63	25	34.19
TSW LR59	67	14.82	0.61	4.96	5.17	10.62	14.14	18.52	29.81

For agronomic traits

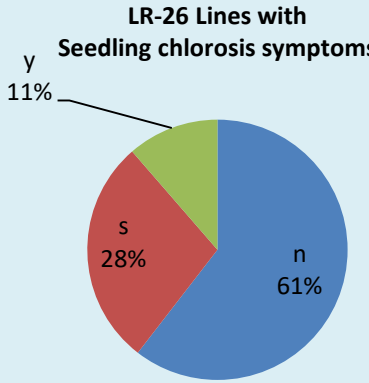


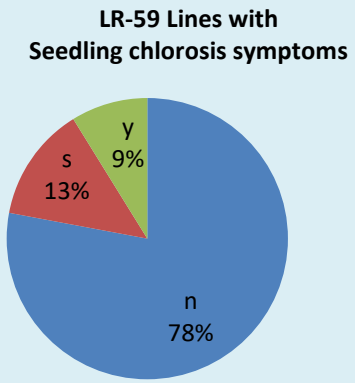






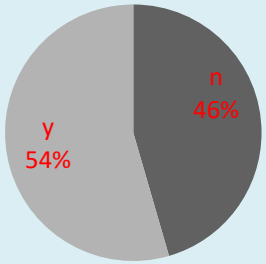
Negative effects

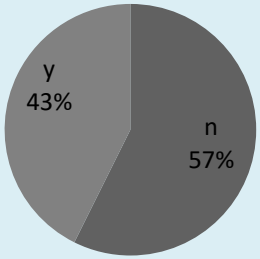




LR-26 Dehiscence

LR-59 Dehiscence





On-going wild lentil introgression studies

- Mapping introgression using LR26 and LR59
- Improving bio-fortification and stress tolerance.
- Using other wild species to expand genetic base for future genetic gain in lentil breeding.

Inter-specific	# RILs		Intra-specific	# RILs
L.c. * *L.ori.*	8		*L.e.* * *L.e.*	1
L.c. * *L.ode.*	3		*L.ori * L.ori.*	3
L.c. * *L.t.*	3		*L.tom * L.tom*	2
L.c. * *L.e.*	2		*L.ode * L.ode.*	1
*L.c * L.l.*	1		*L.l * L.l*	1
*L.t * L.ori.*	1		*L.n * L.n*	1
*L.ori. * L.t.*	1			

Thank you for the attention.

Questions?

University of Saskatchewan

Saskatchewan Pulse Growers

Agriculture Council of Saskatchewan Inc.